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ABSTRACT

Introduction/Purpose To investigate whether a) lower levels of daily physical activity (PA) and greater sedentary time accounted for contrasting metabolic phenotypes (higher liver fat/presence of metabolic syndrome [MetS+] vs lower liver fat/absence of metabolic syndrome [MetS-]) in individuals of similar BMI and b) the association of sedentary time on metabolic health and liver fat. **Methods** Ninety-eight habitually active participants (53 female, 45 male; age 39 ± 13 years; BMI 26.9 ± 5.1 kg/m²), underwent assessments of PA (SenseWear armband; wear time ~98%), cardio-respiratory fitness ($\dot{V}O_2$ peak), body composition (MRI and MRS) and multi-organ insulin sensitivity (OGTT). We undertook a) cross-sectional analysis comparing four groups: non-obese or obese, with and without metabolic syndrome (MetS+ vs MetS-) and b) univariate and multivariate regression for sedentary time and other levels of PA in relation to liver fat. **Results** Light, moderate and vigorous PA did not account for differences in metabolic health between individuals, whether non-obese or obese, although MetS+ individuals were more sedentary, with a higher number, and prolonged bouts (~1-2 hours). Overall, sedentary time, average daily METS and $\dot{V}O_2$ peak were each independently associated with liver fat percentage. Each additional hour of daily sedentary time was associated with a 1.15% (95% CI, 1.14–1.50%) higher liver fat content. **Conclusions** Greater sedentary time, independent of other levels of PA, is associated with being metabolically unhealthy; even in habitually active people, lesser sedentary time, and higher cardio-respiratory fitness and average daily METS is associated with lower liver fat. **KEY WORDS:** Body composition, magnetic resonance spectroscopy, metabolic syndrome, insulin regulation, cardio-respiratory fitness, metabolic equivalents

INTRODUCTION

Strong epidemiologic evidence suggests an inverse relationship between physical activity (PA) levels and obesity, metabolic syndrome (MetS), non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes (1-5). Increased PA is recommended both for individuals and at a population level to improve metabolic health and help prevent these interrelated conditions. The independent protective effect of high cardio-respiratory fitness (CRF), an objective marker of PA, against all-cause mortality is well established (6, 7). There is a growing recognition that sedentary behaviour, which has an independent association with adverse health outcomes, should be minimised (2, 8, 9). Increasing moderate PA is protective against the aforementioned diseases and attenuates, but does not eliminate, the detrimental effects of sedentary behaviour (10). Breaking up prolonged periods of sedentary time (11) or replacing it with low-intensity PA (12) are beneficial for glycaemic control.

Obesity is strongly associated with poor cardio-metabolic health and overall mortality (13). However, not all obese individuals are *metabolically unhealthy* (MetS+) (14); conversely not all non-obese individuals are *metabolically healthy* (MetS-) (15). Some studies suggest that MetS+ may be a consequence of low PA (16, 17), but others have not supported this conclusion (18-20). With differences in methodology, cohort characteristics and definitions of metabolic phenotypes, these studies typically have not precisely defined the differences in PA characteristics between phenotypes. Only one study, of older adults, has *objectively* measured sedentary behaviour (19), which offers better reliability than self-report (21); no such study has been undertaken in young-middle aged adults. There are similarly conflicting results in studies of the association of metabolic health with objectively measured sedentary behaviour and quantitative measures of

liver fat using magnetic resonance spectroscopy (MRS) or computed tomography (CT) (22-26). The accumulation of liver fat has been described as a major contributor to the development of type 2 diabetes (27), is considered the hepatic manifestation of MetS and closely linked with obesity and insulin resistance (28). Observing levels of PA, including sedentary behaviour, in metabolic phenotypes of a given BMI category with further quantification of liver fat may reveal associations which link habitual activity to health outcomes and the predisposition for metabolic diseases.

This cross-sectional study will objectively monitor the habitual PA of young-middle aged adults and extensively phenotype these individuals by assessment of metabolic health and MRI-derived body composition. We hypothesise that, greater sedentary time and lower levels of PA will be evident in metabolically unhealthy phenotypes (MetS+ vs MetS-) in BMI-matched individuals; and secondly, higher MRS-quantified liver fat will be associated with greater sedentary time and lower PA levels.

METHODS

Participants

Habitually active individuals who engaged in no more than 2 hours of exercise per week, were recruited via local advertisements across University of Liverpool campuses and hospital departments. Exclusions included cardiovascular, respiratory, kidney, liver and/or endocrine complications, smoking and >14 units/week of alcohol consumption. The study conformed to the *Declaration of Helsinki* and was approved by the North West Liverpool Central research ethics committee (14/NW/1145; 14/NW/1147; 15/NW/0550). All participants were informed of the

methods verbally and in writing before providing written informed consent prior to any assessments. Ninety-eight individuals (52 male, 46 female) with a mean age of 39 ± 13 years and BMI 27 ± 5 kg/m² were recruited. Prior to each study visit, participants were required to fast overnight for 12 hours (water was permitted *ad libitum*), abstain from alcohol and caffeine for 24 hours and from exercise for 48 hours.

Study design

All participants completed measurement of baseline PA and dietary consumption over a period of 4 days (including one weekend day) between January 2016 and February 2017 followed by assessment in the the order of a) anthropometry (including bio-impedance), fasting biochemistry, an oral glucose tolerance test (OGTT) and assessment of CRF ($\dot{V}O_2$ peak) at University Hospital Aintree and b) magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (¹H-MRS) at the University of Liverpool MRI Centre (LiMRiC). Due to MRI scanner replacement during part of this study, MRI quantification of body fat was conducted in only 72 individuals. Bio-impedance data was collected in all individuals, and $\dot{V}O_2$ peak calculations were based on both total body mass and fat free mass (FFM).

Individual phenotyping

Individuals were characterised into one of four groups based on BMI (non-obese <30 vs obese ≥ 30 kg/m²) and the presence or absence of MetS according to International Diabetes Federation (IDF) criteria; we refer to these groups as i) 'non-obese MetS-', ii) 'non-obese MetS+', iii) 'obese MetS-' and iv) 'obese MetS+'.

Habitual assessment

Physical activity monitoring PA was monitored throughout using a validated (29) SenseWear mini armband (BodyMedia Inc., Pittsburgh, PA, USA). Wear time (recorded as ~98%) was monitored using SenseWear Professional software (version 8.0). Data included: daily average step count, total energy expenditure, active energy expenditure and time spent in levels of PA including: sleep, lying down, sedentary (<1.5 METS), light (1.5-3 METS), moderate (3-6 METS), vigorous (6-9 METS) and very vigorous (>9 METS). A Microsoft Excel template, as previously described (30), was used to determine how sedentary time (not including sleep) was accumulated and provided information on the frequency of bouts and the time accumulated in a given bout category (<1 h: 1–5, 6–10, 11–20, 21–40, 41–60 min; 1–2 h: 61–80, 81–100, 101–120 min; >2 h: 121–140, 141–160, 161–180 min). To examine ‘frequently broken’ periods of sedentary time, the given bout categories at the lower end (<1 h) were shorter in duration. At the higher end (> 1h), where fewer bouts are recorded, the given bout categories are greater in duration. Based on previous observations (31), this approach was adopted to investigate ‘patterns’ of sedentary time, i.e. the frequency with which sedentary time is interrupted (sedentary breaks) or the duration of uninterrupted periods of sedentary time (sedentary bouts). Furthermore, moderate-vigorous PA (MVPA) of bouts greater or less than 10 minutes were determined.

Dietary analysis Total energy consumption, carbohydrate, protein and fat content were determined from 4-day dietary records by a registered nutritionist (KM) using Nutritics (Nutrition Analysis Software for Professionals; <https://www.nutritics.com/p/home>).

Other assessment measures

Anthropometric measurements Stature (Model 220, Seca, Germany) and whole-body bio-impedance analysis (Tanita, BC 420, Dolby Medical Stirling, UK) was conducted; this provided total body mass, fat percentage, fat mass, fat free mass, muscle mass, total body water, basal metabolic rate, bone mass and visceral fat indicator. Waist and hip circumference measurements were taken in duplicate and blood pressure was determined from an average of three measures (Dinamap, G & E Medical, USA).

Biochemical measurements Blood samples were collected and analysed using the Olympus AU2700 analyser (Beckman Coulter, High Wycombe, UK) with standard proprietary reagents as follows: glucose with hexokinase, total cholesterol and HDL with cholesterol esterase/oxidase and triacylglycerol with glycerol kinase. LDL was calculated according to the Friedwald formula. Insulin was measured using radio-immunoassay (Invitrogen, UK). HOMA-IR was calculated using fasting glucose and insulin concentrations.

OGTT Following a 12hr fast, blood samples were collected, a 75 g glucose drink was consumed within 5 min and post-ingestion blood samples were drawn at 30, 60, 90 and 120 min. Matsuda index was calculated to estimate whole body IS, and indices of hepatic-IR and skeletal muscle IS were determined as previously described (32).

CRF A $\dot{V}O_2$ peak cardio-pulmonary exercise test (CPET) was performed on a treadmill (Model 770CE, RAM Medisoftware Group, Manchester, UK) in a temperature-controlled room. The CPET provided breath-by-breath monitoring and analysis of expiratory gases and ventilation (Love Medical Cardiopulmonary Diagnostics, Manchester, UK). The modified Bruce protocol was

employed, after an initial 2 min warm up at 2.2 km/h on a flat gradient, step-wise increments in speed and gradient were employed each minute. $\dot{V}O_2$ peak was determined by exhaustion plus one or more of: respiratory exchange ratio >1.15, heart rate >90% predicted maximum, plateau in $\dot{V}O_2$.

1H -MRS Liver and skeletal muscle fat were determined using a 1.5 T Siemens Symphony MRI scanner as previously described (33).

Statistical analysis

All data were explored for normality using visual inspection of frequency distribution, and logarithmically transformed where appropriate. Given the small sample size, power achieved on each test was assessed and ranged from 46 to >99%; 20 of 26 achieved at least 80% power. Age was analysed using a one factor between-groups ANOVA whereby a significant group effect was observed ($P<0.05$). Between-group univariate general linear models (GLM) were conducted for all other variables, with age as a covariate and Bonferroni correction for multiple comparisons. Statistically significant interactions were explored and nominal P -values reported. Univariate and multivariate linear regression were used to analyse components of PA and fitness associated with liver fat. Decisions were made *a priori* to include all variables reaching $P<0.1$ in univariate regression analysis alongside age and BMI in the multivariate regression model. The statistical cut-off for inclusion in the final model is more stringent than often used to guard against false discovery. The alpha level of statistical significance was set at $P<0.05$. Data are presented as mean (95% CI), unless stated otherwise. Transformed data were back-transformed to original units. P value >1 rounded to 1.000.

RESULTS

Participant characteristics

The numbers of individuals with each risk factor of MetS are summarised in Table 1, with the PA and CRF data of the whole cohort combined. Calculated from their average of 4 d MVPA (accumulated in bouts of >10 min), 61% of individuals met the World Health Organisation (WHO) recommendations.

Metabolic phenotyping

The significant differences between the groups' components of MetS were in line with IDF classification (Table 2). There was no significant difference between obese MetS- and obese MetS+ BMI ($P=0.712$) but non-obese MetS+ BMI was 3 ± 2 kg/m² greater than non-obese MetS- ($P=0.003$). In the general population, MRS defined liver fat >5.5% corresponds with the prevalence of hepatic steatosis (34); 84 and 14 participants had liver fat <5.5% and $\geq 5.5\%$ respectively.

Dietary intake

Total energy consumption, carbohydrate, protein and fat did not differ significantly between groups ($P>0.05$). Mean \pm SD macronutrient percentages were $56\pm 16\%$ carbohydrate, $24\pm 9\%$ protein, and $20\pm 7\%$ fat.

CRF

Obese MetS+ individuals had lower CRF than both obese and non-obese MetS- ($P \leq 0.029$; mean difference $\geq 7.5 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) but not non-obese MetS+ ($P = 0.675$; mean difference $5.9 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$). There was no difference between both non-obese groups and obese MetS- ($P \geq 0.080$) (Fig. 1a).

Multi-organ IS

Non-obese MetS- individuals had greater Matsuda index than non-obese MetS+ ($P = 0.012$; mean difference 2.0) (Fig. 1b); there was no difference between obese MetS- and both MetS+ groups ($P \geq 0.141$). There was no group effect for skeletal muscle IS index ($P = 0.220$). Hepatic-IR index was greater in obese MetS+ than non-obese MetS- (Fig. 1c). There was a significant group effect ($P = 0.022$) for HOMA-IR.

MRS quantification of liver fat

Liver fat was higher in MetS+ in both non-obese and obese. Non-obese MetS- individuals had 4.6% lower liver fat than obese MetS+ ($P \leq 0.005$) (Fig. 1d). Liver fat percentage in non-obese MetS+ was not different to either obese group ($P \geq 0.794$; mean difference $\geq 0.6 \%$); and liver fat percentage in obese groups was not statistically different ($P = 0.336$; mean difference 2.6 %).

Levels of physical activity: differences between the 4 metabolic phenotypes

Average daily steps: There was no group effect for average daily steps (Fig. 2a).

Non-sleep sedentary time, lying time and sleep duration: Non-sleep sedentary time (Fig. 2b) was not different between non-obese groups ($P = 1.000$; 49 min/day) and obese groups ($P = 1.000$; 23

min/day). Non-obese MetS- individuals had lower sedentary time than obese MetS+ ($P=0.04$); there was no difference between obese MetS- and both MetS+ groups ($P\geq 0.199$). There was no group effect for amount of time spent lying down ($P=0.080$) or sleeping ($P=0.117$).

Daily light PA time: There was no difference in daily light activity between both non-obese groups ($P=0.711$; mean difference 10 min/day) and both obese groups ($P=1.000$; 9 min/day). However, both obese groups had less light activity than both non-obese MetS- ($P\leq 0.015$; mean difference ≥ 69 min/day) (Fig. 2c).

Daily moderate-vigorous PA time: There was no difference between the groups' moderate-vigorous activity ($P=0.322$) (Fig. 2d) and no significant differences were found for the way in which MVPA was accumulated for bouts of 10 minutes or more, in either total minutes accumulated or percentage of the time in relation to total MVPA.

Average daily METS and PA duration: Daily average METS (Fig. 2e) and PA duration (Fig. 2f) had significant group effects ($P<0.0005$ and $P=0.020$, respectively); for both measures, non-obese MetS- had greater values than both obese groups, but were not different to non-obese MetS+. Daily average METS in non-obese MetS- were 0.3 METS greater than both obese groups ($P<0.0005$). The same was observed for PA duration, with non-obese MetS- having greater duration than both obese groups ($P\leq 0.018$; mean difference ≥ 107 min/day). There was no significant difference between obese MetS- and both MetS+ groups for average daily METS and PA duration ($P\geq 0.079$ and $P\geq 0.450$ respectively).

Patterns of waking sedentary time: Analysis of sedentary behaviour was performed on waking sedentary time examining the *duration of sedentary time* (Fig. 3a) and *the number of sedentary bouts* (Fig. 3b) in a pre-determined bout category. There were no differences between the groups

in sedentary bout durations of < 1 h or > 2 h. However, significant differences were apparent during bout durations lasting *between* 1 and 2 h. *Duration:* during bouts of 61-80 min, non-obese MetS+ accumulated 33 min more sedentary time per day than non-obese MetS- (3, 60; $P=0.013$). During bouts of 81-100 min, MetS+ obese accumulated 34 min per day more than obese MetS- (6, 62; $P=0.018$). During bouts of 101-120 min, obese MetS+ accumulated 28 min per day more than obese MetS- (5, 51; $P=0.018$). *Number of bouts:* as an average of 4 days, both MetS+ groups accumulated 1-2 more long bouts (between 1-2 h) of sedentary behaviour, compared to their MetS- counterparts. Considering bouts of 61-80 min, non-obese MetS+ had 0.5 more bouts per day (0.1, 0.9; $P=0.012$) than MetS-. Obese MetS+ had 0.4 more bouts per day (0.1, 0.7; $P=0.019$) than MetS- of 81-100 min and 0.3 more bouts per day (0.1, 0.5; $P=0.017$) of 101-120 min.

Levels of physical activity (regression analysis): Univariable linear regression analysis revealed that daily average steps, sedentary time, vigorous activity, METS and $\dot{V}O_2$ peak were all significantly associated with liver fat. Carried forward in the multivariable analysis, three of these factors remained statistically significant predictors of liver fat (Table 3). Greater daily sedentary time is associated with higher liver fat, while higher overall daily METS and $\dot{V}O_2$ peak are associated with lower liver fat (Fig. 3). For a one hour increase in sedentary time, liver fat increased by 1.15% (1.14–1.50%; $P=0.036$) while for a $1 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$ increase in CRF ($\dot{V}O_2$ peak), liver fat reduced by 0.87% (0.25, 1.50; $P=0.007$).

DISCUSSION

The results of this extensive phenotypic analysis of objective measurements of PA and sedentary behaviour, metabolic and body composition measurements (including MRS-derived liver fat) in

young-middle aged adults demonstrate two key messages. Firstly, in this cohort, overall habitual PA was not associated with different metabolic health status in individuals of similar BMI, and the accumulation of sedentary time was weakly associated with the presence of the MetS. Secondly, even in habitually active individuals, there is an association between greater sedentary time and increased liver fat, while the amount of moderate-vigorous PA (MVPA) appeared to have little independent association. These data highlight the potential importance of sedentary behaviour in determining optimal metabolic health and liver fat.

It is recognised that greater sedentary time increases the risk of becoming overweight/obese (35) and the risk of type 2 diabetes and cardiovascular disease, even after controlling for MVPA (8, 36). Whilst total volumes of habitual PA do not explain metabolic health in this cohort, those with MetS shown some evidence of being more sedentary, with a higher number of prolonged bouts of sedentary behaviour (between 1-2 hours). Frequent breaks in sedentary time have been shown to be beneficial to metabolic risk (31), health (37) and liver fat (24). To our knowledge, there are no studies which have investigated sedentary bouts greater than 1 hour. Interestingly, an extra hour of sedentary time has been associated with a 39% increased odds for MetS (38) and decreasing sedentary time accumulated in prolonged bouts may have beneficial effects on BMI and waist circumference (39). Further research at durations of >1 hour may reveal insight into the pattern in which sedentary time is accumulated and MetS. Even individuals who are physically active can still spend a significant amount of their waking day sedentary, (termed previously *sedentary exercisers*' (40)) which is associated with increased cardio-metabolic risk. Taken together, these findings suggest that public health and chronic disease prevention strategies that largely focus on MVPA recommendations, might benefit from new

recommendations regarding interruption of prolonged sedentary time, complimentary to those of PA.

Numerous prospective studies have confirmed the relationship between PA and liver fat (5, 41-44) and compliance with national MVPA guidelines has been associated with a lower odds of NAFLD (26). Furthermore, recent research in a population-based sample of adults has shown that $\dot{V}O_2$ peak is strongly, inversely and independently related to the risk of liver fat (45). The results presented are in agreement with previous research, greater levels of PA (here daily METs) and higher CRF is independently associated with lower levels of liver fat. Importantly, the associations between CRF and liver fat remained after adjustment for BMI; not all studies have reported similar findings (46). The association between sedentary time and liver fat is equivocal. Some authors have found no associations between PA or sedentary behaviour and liver fat in 82 individuals (25, 26). Whereas others have concluded that PA and sedentary time are indeed independently associated with the prevalence of NAFLD (22, 24). In *inactive* individuals every hour of sedentary time was associated with increases of 1.74 L of total abdominal fat, 0.62 L of visceral fat, 1.14 L of subcutaneous fat, and 1.86% liver fat (22). Direct comparisons or broad conclusions are difficult due to differences in cohorts and methodology. The findings of the current study suggest that sedentary time has an independent effect on liver fat in active adults, however more data is required to confirm this. Our results demonstrating that every hour of additional sedentary time translates to a 1.15% increase in liver fat, can be put into context by comparing the effects of a 4 week aerobic cycling intervention in sedentary obese men and women, where liver fat reduced by 1.7% (47). The effects surgical, nutritional, lifestyle or pharmaceutical interventions aiming to reduce liver fat has been recently reviewed (48).

This study utilises objective monitoring of PA, gold standard measurement of CRF and MRS-derived liver fat in young-middle aged adults, all of which are key strengths. The results did not support any strong evidence for a beneficial association of sedentary bouts <1 hour or detrimental association of >2 hours perhaps due to study limitations which include the relatively small sample size. Further limitations include: duration of PA assessment, the monitor used to assess sedentary behaviour (SenseWear does not determine postural differences), the comparatively healthy habitual PA habits of the participants which somewhat limits the external validity of the findings, and the cross-sectional design which cannot determine causality. Noteworthy is the higher BMI in unhealthy non-obese versus healthy non-obese which confirms to the association of a greater BMI with greater metabolic risk. This difference could not be controlled for as it was a component of our grouping analysis but differences in age were statistically controlled for. While the present results demonstrate that overall sedentary time needs to be considered independently of PA, objective PA monitoring in a larger cohort with a prospective design will be required and future research should further explore sedentary behaviour patterns (i.e. amount of sedentary breaks and duration of sedentary bouts). The American Diabetes Association has recommended that adults should '*decrease the amount of time spent daily in sedentary behaviour*' and that '*prolonged sitting should be interrupted with bouts of light activity every 30 min*'. Importantly, these recommendations are in addition to, not a substitute for, a physically active lifestyle. A 'cut-off' for harmful sedentary behaviour patterns (i.e. frequency/duration) has not been defined in public health guidelines.

In summary, in habitually active adults, the amount of sedentary time is associated in this single-measure observation with metabolic health and the quantity of liver fat. The findings of this

study highlight that public health policy designed to optimise the benefits of physical activity may need to synergistically consider strategies to reduce sedentary behaviour.

ACCEPTED

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Conflict of Interest

The authors declare that there is no conflict of interest associated with this manuscript. MH has support from NIHR Leicester BRC. The results of the study do not constitute endorsement by ACSM and are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Author contribution statements

DJC, GJK and JPHW conceived the study or parts of the study. KBD, VSS, JAN, DJC, generated the data. GJK analysed the MRS data. KLM analysed the nutritional data. KBD and AT statistically analysed and interpreted the data. All authors participated in preparation of the manuscript and approved the final version for publication.

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Figure legends

Figure 1 Cardio-metabolic phenotyping, individual participant plots for: $\dot{V}O_2$ peak relative to fat free mass (FFM) (a), whole body insulin sensitivity (b), hepatic insulin resistance index (c) and liver intrahepatocellular lipid (IHCL) (d). Data are presented as mean \pm SD. Grey circles, MetS-; white circles, MetS+; non-obese are grouped left and obese are grouped right. * $P < 0.05$ group difference between BMI category, further group differences being given in the text.

Figure 2 Habitual physical activity and sedentary time, individual participant plots for: average daily steps (a), non-sleep sedentary time (< 1.5 METS) (b), light activity ($1.5-3$ METS) (c), moderate-vigorous activity (> 3 METS) (d), daily metabolic equivalents (METS) (e) and physical activity (PA) duration (f). Data are presented as mean \pm SD. Grey circles, MetS-; white circles, MetS+; non-obese are grouped left and obese are grouped right. * $P < 0.05$ group difference between BMI category, further group differences being given in the text.

Figure 3 Non-sleep sedentary behaviour, individual participant plots for: duration of sedentary bouts (a) and number of sedentary bouts in given bout category (b) between 1-2 h. Data are presented as mean \pm SD. Grey circles, MetS-; white circles, MetS+; non-obese are grouped left and obese are grouped right. * $P < 0.05$ group difference between BMI category, further group differences being given in the text.

Figure 1

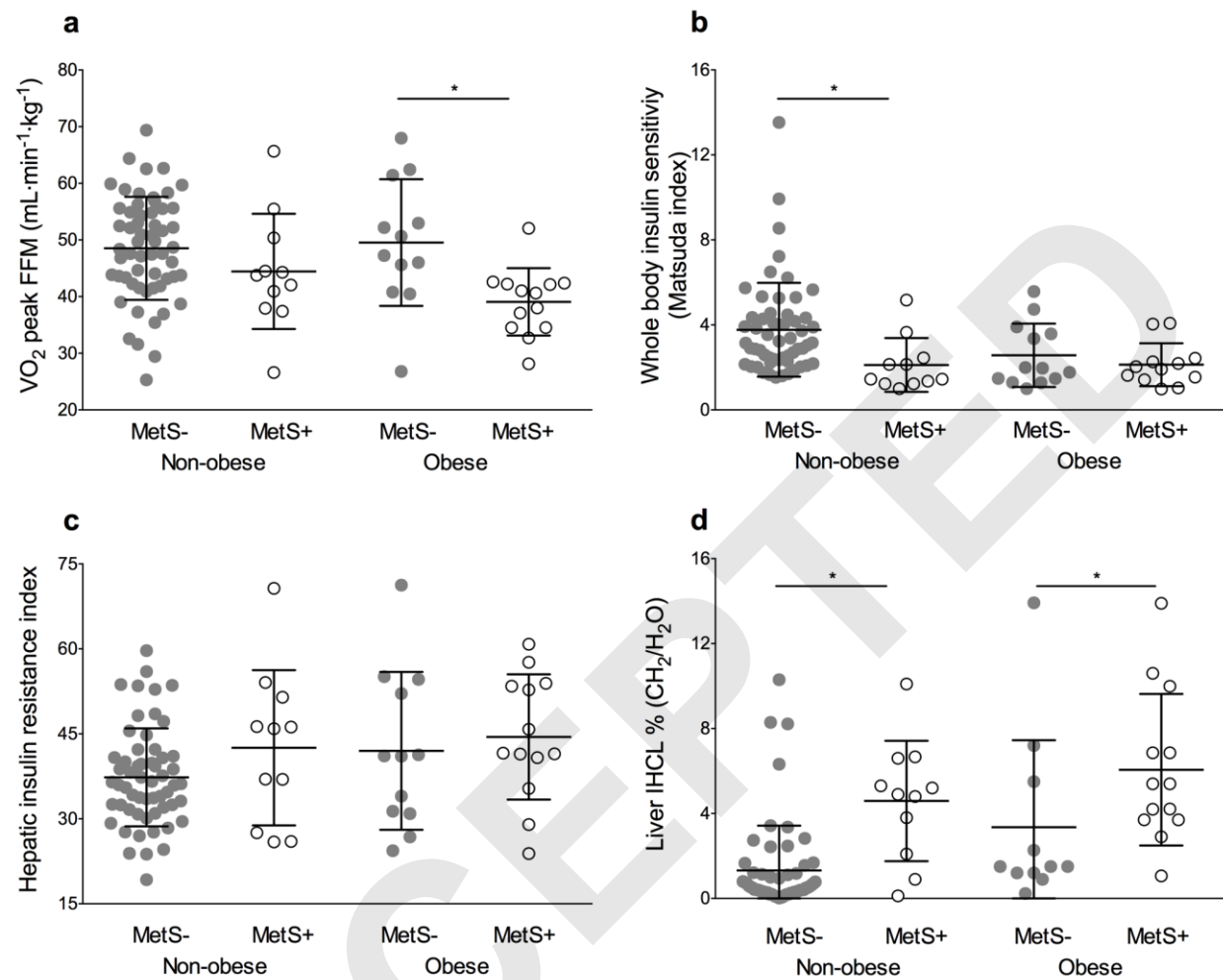


Figure 2

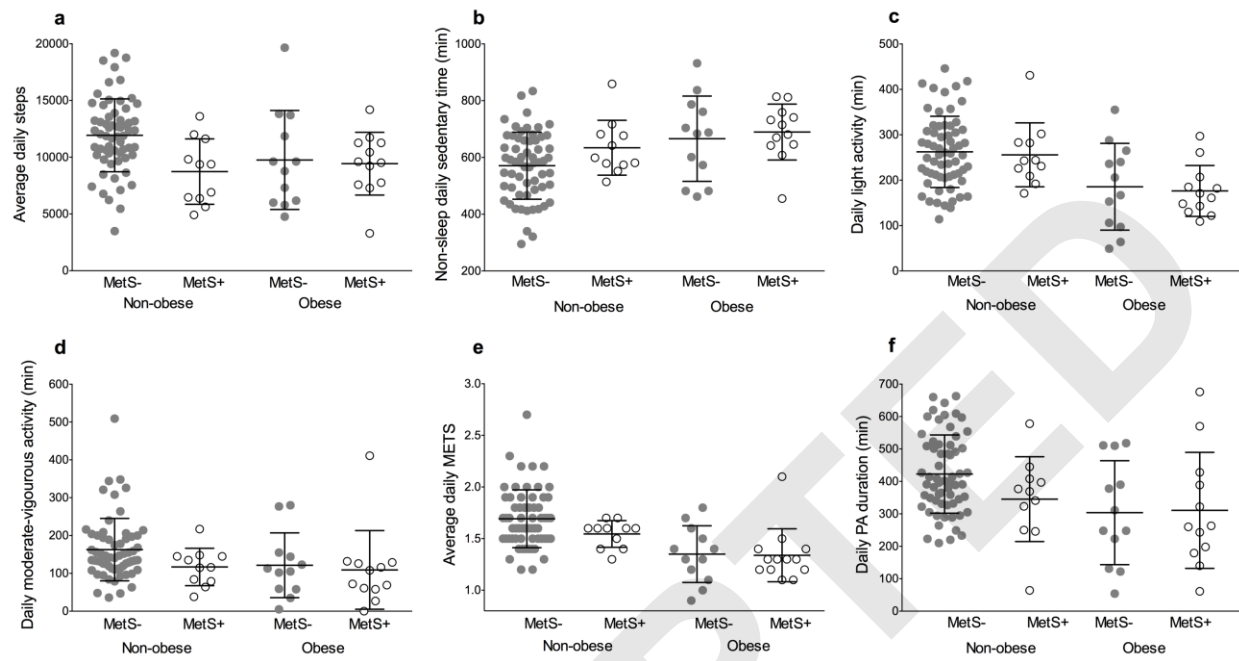


Figure 3

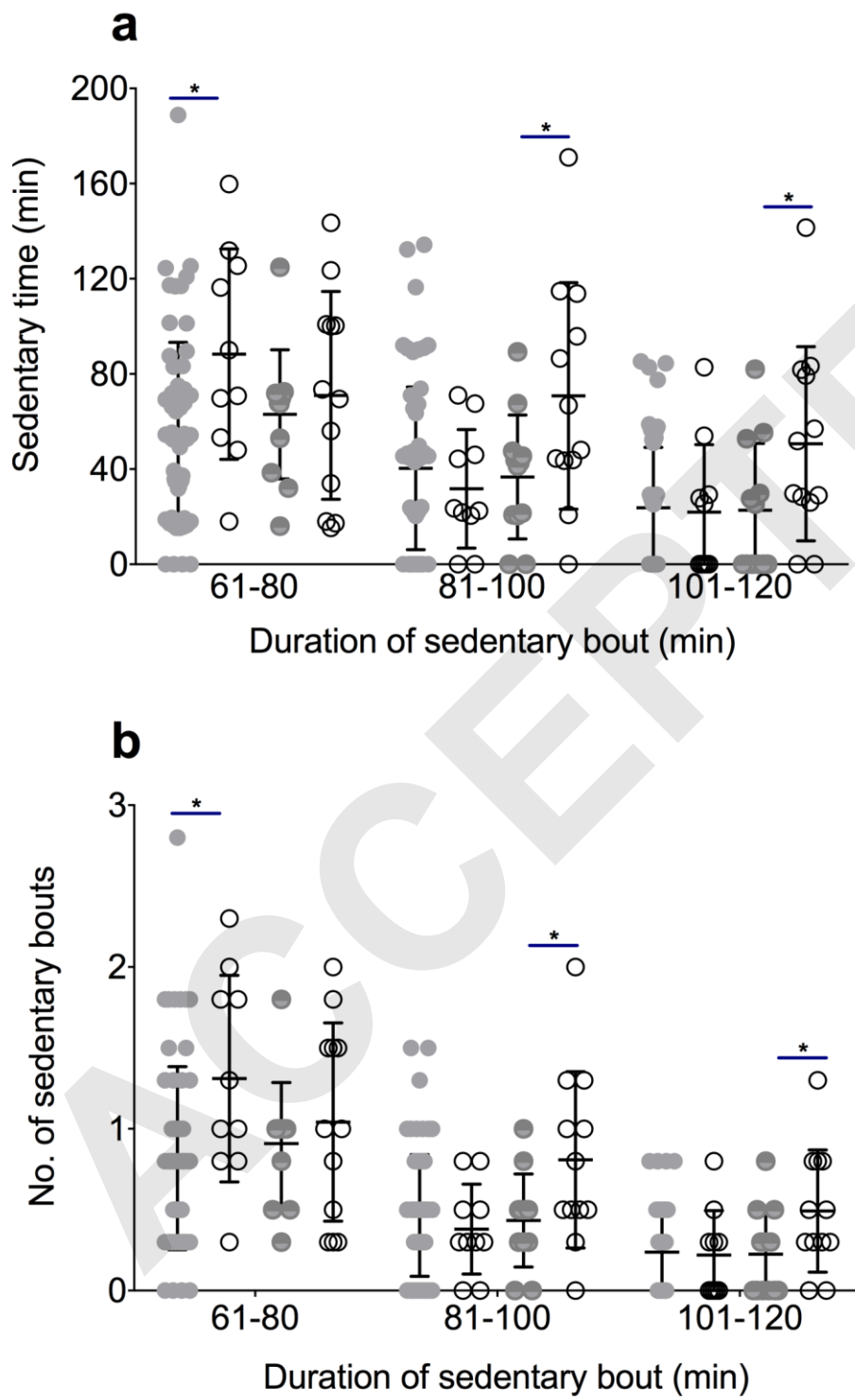


Table 1 PA and CRF data, the number of risk factors of metabolic syndrome (MetS) and liver fat in 98 individuals. Classification column for risk factors is listed as MetS- (top) and MetS+ (bottom).

	Mean \pm SD	
Average daily wear time (%)	98 \pm 4	
Average daily steps (steps/day)	10939 \pm 3482	
Daily non-sleep sedentary time (min)	605 \pm 125	
Daily light PA time (min)	241 \pm 84	
Daily moderate-vigorous PA time (min)	143 \pm 92	
Daily lying time (min)	486 \pm 83	
Daily sleep duration (min)	403 \pm 67	
Daily metabolic equivalents (METS)	1.6 \pm 0.3	
$\dot{V}O_2$ peak (mL \cdot min ⁻¹ \cdot kg ⁻¹)	32.9 \pm 8.2	
Risk factor	Classification	N (%)
Waist circumference (cm)	<94M/80F	65 (66%)
	\geq 94M/80F	33 (34%)
Triacylglycerol (mmol/L)	\leq 1.7	83 (85%)
	>1.7	15 (15%)
HDL-cholesterol (mmol/L)	\geq 1.03M/1.29F	91 (93%)
	<1.03M/1.29F	7 (8%)
Systolic BP (mmHg)	\leq 130	64 (65%)
	>130	34 (35%)

Diastolic BP (mmHg)	≤85	74 (76%)
	>85	24 (24%)
Fasting glucose (mmol/L)	≤5.6	88 (90%)
	>5.6	10 (10%)

PA, physical activity; HDL, high-density lipoprotein; BP, blood pressure; M, male classification; F, female classification.

Table 2 Clinical, metabolic and body composition characteristics of participants categorised for obesity and subsequently according to MetS.

	Non-obese			Obese		
	MetS- (n=62)	MetS+ (n=11)	P	MetS- (n=12)	MetS+ (n=13)	P
Gender	M n=30; F n=32	M n=9; F n=2	0.042*	M n=5; F n=7	M n=8; F n=5	0.319
Age (years)	34 (31, 38)	49 (43, 55)	<0.0005*	45 (39, 50)	46 (39, 52)	0.902
Weight (kg)	70.8 (68.1, 73.6)	80.8 (75.7, 85.9)	0.045*	96.3 (85.2, 107.4)	99.8 (91.7, 107.9)	0.470
BMI (kg/m ²)	24.1 (23.4, 24.8)	26.9 (25.7, 28.2)	0.018*	33.7 (30.6, 36.7)	34.1 (32.6, 35.6)	0.722
Components of metabolic syndrome						
Waist circumference (cm)	85 (82, 87)	98 (93, 102)	0.005*	105 (96, 115)	111 (106, 116)	0.191
Systolic BP (mmHg)	120 (117, 123)	144 (137, 151)	<0.0005*	126 (117, 135)	147 (135, 158)	<0.0005*
Diastolic BP (mmHg)	75 (72, 77)	95 (85, 105)	<0.0005*	77 (73, 80)	90 (82, 98)	0.001*
Fasting glucose (mmol/L)	4.9 (4.8, 5.0)	5.4 (5.1, 5.6)	0.076	5.0 (4.7, 5.2)	5.7 (5.0, 6.4)	0.003*
Triacylglycerol (mmol/L)	0.9 (0.8, 1.1)	1.5 (1.0, 1.9)	0.080	1.2 (0.6, 1.7)	1.8 (1.3, 2.4)	0.016*
HDL-cholesterol (mmol/L)	1.8 (1.7, 1.9)	1.7 (1.2, 2.1)	0.527	1.6 (1.3, 2.0)	1.3 (1.6, 1.8)	0.133
MRI derived body composition	n=48	n=8		n=8	n=8	

Total body fat (L)	21.3 (18.9, 23.7)	25.8 (20.1, 31.5)	0.164	39.6 (33.6, 45.6)	39.1 (33.2, 44.7)	0.882
Total SAT (L)	16.5 (14.2, 18.8)	18.6 (13.1, 24.1)	0.492	30.5 (24.7, 36.3)	28.2 (22.7, 33.8)	0.562
Total internal fat (L)	4.7 (4.1, 5.4)	7.3 (5.7, 8.9)	0.006*	9.2 (7.5, 10.9)	8.5 (6.9, 10.2)	0.552
Abdominal SAT (L)	4.5 (3.5, 5.5)	5.7 (3.3, 8.1)	0.374	9.7 (7.2, 12.3)	12.1 (9.7, 14.6)	0.162
VAT (L)	2.3 (1.9, 2.8)	4.2 (3.1, 5.2)	0.002*	5.2 (4.1, 6.2)	5.7 (4.5, 6.8)	0.490
VAT: abSAT ratio	0.6 (0.5, 0.7)	0.7 (0.5, 0.9)	0.333	0.6 (0.4, 0.8)	0.6 (0.4, 0.8)	0.793

Data shown are mean (95% CI) and *P* values between groups

**P*<0.05

SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue; abSAT, abdominal SAT

Table 3 Multivariate regression for liver fat percentage (%).

	β coefficient	95% CI	<i>P</i>
Liver fat %			
Age (years)	1.00	1.00, 1.02	0.343
BMI (kg/m ²)	1.01	0.97, 1.12	<0.0005*
Average daily steps (1,000)	-0.97	-0.89, -0.97	0.103
Average daily sedentary time (h)	1.15	1.14, 1.50	0.036*
Average daily vigorous activity (min)	-0.01	-0.01, 0.01	0.273
Average daily METS (0.1)	-0.48	-0.13, -0.56	0.012*
$\dot{V}O_2$ peak (mL·min ⁻¹ ·kg ⁻¹)	-0.87	-0.25, -1.50	0.007*

Liver fat data was transformed and analysed using \log_{10} , the data presented here is back transformed to original units.

* $P < 0.05$